the intracellular Ca^{2+} concentration, which in turn would activate a Ca^{2+}-activated neutral proteinase (CANP). A CANP has been described in mammalian skeletal muscle[6,7]; also, a CANP with higher than normal activity has been reported in dystrophic hamsters and mice[9]. However, the role of a CANP in degradation of muscle proteins is still not clear. A likely role for CANP would be to initiate myofilament degradation which would then be followed by activation of lysosomal enzymes.

The involvement of lysosomal enzymes in the action of the ionophore A23187 has been inferred from experiments in which the lysosomal thiol protease inhibitor leupeptin has been used. Thus, Rodemann et al.[4] showed that the increase in protein degradation induced by A23187 in mammalian skeletal muscle can be prevented by pretreatment with leupeptin. Furthermore, the same authors reported that the increase in lysosomal enzymes, specifically cathepsin B, is mediated by prostaglandins. In fact, in rat skeletal muscle A23187 increased the release of prostaglandins E_2 and $F_{2\alpha}$ 3–4 fold. There is, however, no direct evidence indicating that the ionophore increases the activity of lysosomal enzymes.

The results reported here, and summarized in the table, indicate that the morphological damage produced by the ionophore A23187 in frog skeletal muscle is mediated by prostaglandins. Since prostaglandins are widely distributed in the animal kingdom[10,11], it should come as no surprise to find them in frog skeletal muscle. With all the inhibitors of prostaglandin used, and also leupeptin, there was a significant difference in the time necessary to initiate fiber breakdown. Furthermore, once breakdown had started it progressed much slower in preparations preincubated with the inhibitors than in control preparations. It is clear, however, that damage could not be totally prevented even when Ca^{2+} was removed from the incubation medium. It is quite possible that the ionophore is able to release Ca^{2+} from intracellular stores, as it has been shown in other systems[12–14].

Rodemann et al.[4] postulated that cathepsin B and not a CANP is responsible for the increase in protein degradation induced by A23187 in rat skeletal muscle. Their conclusion was based on the fact that the sulfhydryl inhibitor mersalyl, while it completely inhibited the CANP, did not prevent the increase in protein breakdown. In the present work, experiments conducted with mersalyl proved inconclusive. Addition of mersalyl (200 μM) to the incubating bath produced muscle twitching which sometimes led to fiber damage. This effect of mersalyl is mediated by excitation of the nerve terminals since it can be prevented by preincubation with d-tubocurarine. In fact, Binah et al.[15] have shown that mersalyl, as well as other mercurials, greatly increases the frequency of the spontaneous miniature endplate potentials in the frog neuromuscular preparation in vitro.

The present results provide evidence for a role of prostaglandins in skeletal muscle fiber destruction induced by Ca^{2+}. Nevertheless, a direct action of the ionophore on the muscle enzymatic systems, CANP or lysosomes, cannot be ruled out at this time. These results are important in the context of trying to find mechanisms that can minimize the morphological alterations that occur in skeletal muscle in different pathological conditions.

1 The author would like to thank Dr R. J. Walter for helpful comments and Ms V. Kriho for valuable technical assistance.

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0014-4754/85/121551-02$1.50 + 0.20/0

Beta-adrenergic-stimulated adenylate cyclase activity in normal and EBV-transformed lymphocytes

R. P. Ebstein, M. Steinitz, J. Mintzer, I. Lipshitz and J. Stessman

Jerusalem Mental Health Center-Ezrath Nashim, Department of Geriatric Research, P.O.B. 140, Jerusalem (Israel), and Hadassah University Hospital, Department of Hematology, Mount Scopus, Jerusalem (Israel), 10 December 1984

Summary. Beta-adrenergic-associated cyclic AMP accumulation was studied in intact lymphocytes before and after transformation with Epstein-Barr virus into immortal cell lines. Although a marked reduction in isoproterenol-stimulated cyclic AMP synthesis was observed in transformed cells, forskolin-stimulated cyclic AMP accumulation was preserved. A parallel loss of ^{125}-iodocyanopindolol binding sites suggests that the reduction in beta-adrenergic-stimulated AMP synthesis is due to receptor down regulation.
Key words. Epstein-Barr virus; beta-adrenergic receptors; cyclic AMP synthesis; forskolin; adenylate cyclase; lymphocytes.

Epstein-Barr virus (EBV)-transformed lymphocytes are a powerful tool in immunology and virology since EBV virus induces unlimited growth of human B cells with an extremely high efficiency[2], concomitantly with preservation of the differentiated state as evidenced by the prolonged in vitro secretion of specific monoclonal antibodies[3,4]. Although specific immunological characteristics are maintained by transformed or immortal cell lines[3,4], less is known about the fate of other lymphocyte functions after EBV transformation. Such non-immunological functions are of particular interest, however, in investigations that use transformed lymphoblasts as a model system for studying the interaction of environment and heredity in the etiology of various disease states[5–7]. Circulating lymphocytes, including both B and T cells, possess a beta-adrenergic receptor linked to adenylate cyclase, which has been the subject of extensive clinical and biochemical investigations[8–11]. The presence of a well-defined adenylate cyclase complex in circulating lymphocytes and the demonstrated importance of cyclic AMP in a variety of cell processes prompted us to follow changes in this system subsequent to EBV transformation and growth in culture. The

results from this study could be helpful by: a) defining the usefulness of transformed lymphoblasts in studies of hormone-linked adenylate cyclase function in man and b) in elucidating the little-understood mechanisms involved in virus-induced lymphocyte transformation[12].

Materials and methods. Lymphocytes were isolated from 16 individual donors and a portion of the cells were used to generate EBV-transformed cell lines. Cyclic AMP accumulation in the remaining non-transformed lymphocytes was measured after stimulation with isoproterenol, a beta-adrenergic receptor agonist, and with forskolin, a unique diterpene which has been shown to activate adenylate cyclase by direct action on the catalytic subunit of the enzyme-receptor complex[13]. The transformed cells were grown in culture and cyclic AMP accumulation was then measured in the presence of isoproterenol and forskolin. Lymphocytes were isolated from heparinized blood using the method of Boyum[14]. EBV-immortalized cell lines were established as previously described[3,4].

Cyclic AMP was determined as follows: After 30 min preincubation at 37 °C in a balanced salt solution, pH 7.6, 1-ml aliquots of the cell suspensions were incubated for an additional 10 min in the presence or absence of either L-isoproterenol or forskolin.

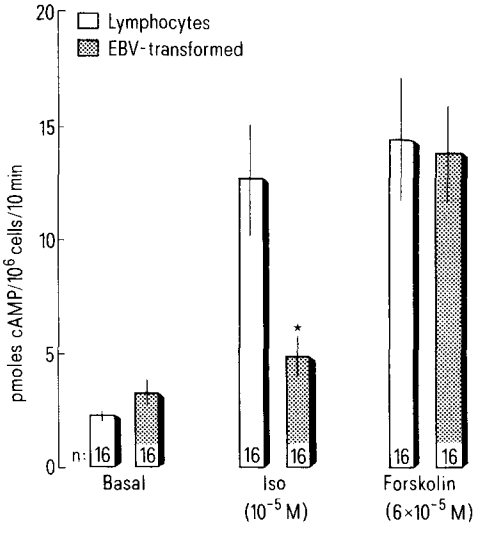

Figure 1. Cyclic AMP accumulation in normal lymphocytes and in EBV-transformed cells. *$p < 0.005$ (paired t-test, comparing isoproterenol-stimulated cyclic AMP synthesis before and after EBV-transformation for each individual donor). Bars are mean values ± SEM.

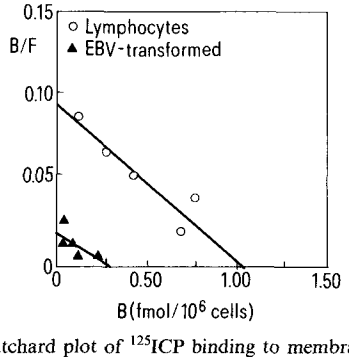

Figure 2. Scatchard plot of ^{125}ICP binding to membranes from human lymphocytes and EBV-transformed cell lines. The ratio B/F of specifically bound ^{125}ICP (fmol/10^6 cells) to free ^{125}ICP (pM) is plotted as a function of B, specifically bound ^{125}ICP. The points are the average values from four experiments and the line is the best fit determined by linear regression analysis.

IBMX (0.25 mM), a potent phosphodiesterase inhibitor, was present in the reaction mixture. Cells were collected by centrifugation and disrupted with a polytron homogenizer after the addition of 1 ml of ethanol; cyclic AMP was determined by a protein binding assay[15]. The concentrations of forskolin and isoproterenol employed had previously been shown to give maximal stimulation of cyclic AMP synthesis under these conditions[8,9].

Binding of ^{125}ICP (Amersham, sp.act. > 2000 Ci/mmol) was carried out according to the method of Brodde et al.[16]. Cells were suspended in ice-cold distilled water, disrupted with a polytron homogenizer, centrifuged at 50,000 × g for 30 min and after one wash resuspended in 10 mM Tris buffer (pH 7.5) containing 5 mg/l BSA and 0.14 M NaCl. The membrane suspension was incubated with various concentrations (ranging from 20 to 400 pM) of ^{125}ICP (Amersham) in the presence and absence of 10^{-5} M propranolol for 1 h at 37 °C, and then collected by rapid filtration onto GF/C filters and washed twice with 3 ml warm buffer.

Observations and discussion. A marked reduction in isoproterenol-stimulated cyclic AMP accumulation was observed in EBV-transformed cells in comparison to normal lymphocytes (fig. 1). On the other hand, no significant changes in forskolin-stimulated cyclic AMP synthesis or basal cyclic AMP levels were observed. Since forskolin stimulates adenylate cyclase by bypassing the receptor and directly affecting the catalytic subunit[13], the results in figure 1 suggested that after EBV transformation there was a deterioration in function of the receptor component of the beta-adrenergic-associated adenylate cyclase complex. In order to test this possibilitiy, lymphocytes and immortal cell lines from 4 donors were studied more intensively: specific binding of ^{125}-iodocyanopindolol (^{125}ICP) to cell membrane isolated from lymphocytes and EBV-transformed cells was examined. The results, presented as Scatchard plots[17], are shown in figure 2. A marked reduction in antagonist binding was observed in membranes obtained from immortal cell lines compared to normal lymphocyte membranes: $B_{max} = 0.29$ fmol vs $B_{max} = 1.03$ fmol ^{125}ICP bound per 10^6 cells. A somewhat decreased affinity of ^{125}ICP for membranes from the immortal cell lines compared to normal lymphocytes was also observed: $K_D = 10.9$ vs $K_D = 17.6$ pM. These changes in ligand-binding properties of EBV-transformed cell membranes explain the observed reduction in isoproterenol-stimulated cyclic AMP accumulation in transformed cells.

The use of cell culture to analyze human disease including diseases of the central nervous system is increasingly recognized as a powerful technique in the investigation of human pathology[5–7]. Although EBV-transformed human lymphoblasts have been used to investigate beta-adrenergic-stimulated adenylate cyclase function in some diseases[18], the marked loss of beta-adrenergic receptors as a result of virus infection and subsequent growth in culture complicates interpretation of such studies. On the other hand, the preservation of forskolin-stimulated activity makes it possible to investigate changes in N protein/catalytic subunit activity such as have been suggested to occur during human aging[8–10] and in some endocrinological disorders such as pseudohypoparathyroidism[7].

The mechanism by which EBV enables lymphocytes to grow indefinitely in culture is not well understood but presumably depends on expression of some of the 50–100 genes encoded by the viral DNA[19]. It is interesting to note that the observed loss of isoproterenol responsiveness after EBV transformation reduces the ability of the transformed cell to respond to hormones that normally raise intracellular cyclic AMP levels. Cyclic nucleotides play an important role in many cell processes including cell division[20], and a number of agents which raise intracellular cyclic AMP levels have been shown to inhibit immunological function and mitogenesis in lymphocytes[21]. Non-viral mitogens may also exert similar effects on receptor function in affected cells. For example, in concanavalin A-stimulated mouse lymphocytes a

decrease in cyclic AMP levels in late G_1 phase is required for the progression from G_1 to S phase[21]. Reduced activity of adenylate cyclase is also observed after stimulation of lymphocytes with the tumor promoter and T-cell mitogen phorbol myristate acetate[22]. These results suggest that different classes of transforming agents also require a reduction in intracellular cyclic AMP levels as an essential permissive condition in the transformation process. It is tempting to speculate that reduced ability of EBV-transformed cells to respond to hormones that stimulate cyclic AMP synthesis is one step in the progression of normal lymphocytes into immortal cells which have escaped from existing growth control mechanisms.

1 Acknowledgment. This work was supported in part by the following organizations: Chief Scientist's Office, Israel Ministry of Health; Herman Goldman Foundation, New York; United Jewish Endowment Fund of Greater Washington – Pollinger Foundation.

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0014-4754/85/121552-03$1.50 + 0.20/0

Occurrence, distribution and nature of neuropeptide Y in the rat pancreas

F. Carlei, J.M. Allen[1], A.E. Bishop, S.R. Bloom and J.M. Polak

Departments of Histochemistry and Medicine, Royal Postgraduate Medical School, Ducane Road, London W12 OHS (England), 12 December 1984

Summary. Significant quantities of a newly discovered peptide, neuropeptide Y, were found in the rat pancreas, where they were localized to nerves in the exocrine parenchyma and around arterial and ductal structures. Although unaffected by surgical parasympathectomy, the periarterial and periductal nerves were abolished by chemical sympathectomy, suggesting that NPY is partially costored with sympathetic transmitters in nerve fibers.
Key words. Immunocytochemistry; neuropeptide Y; radioimmunoassay; rat pancreas.

The characterization of the family of pancreatic polypeptides began in 1974 with the discovery of avian (APP) and bovine (BPP) pancreatic polypeptides[2]. Immunocytochemistry originally localized these peptides to a well-defined endocrine cell type in the islets of Langerhans[3]. However, it was later found that antisera to the PP molecules cross-reacted with central and peripheral nerves[4,5], although the neuronal immunoreactivity could not be detected radioimmunologically[6]. An explanation for this morphologically detectable PP-like substance in nerves came with the recent discovery of NPY (neuropeptide Y), which was isolated from the porcine brain and found to be a member of the PP family of peptides[7,8]. Further studies have revealed a very broad distribution of NPY, not only in the brain[9] but also in most peripheral tissue, including the gut[10-15] and pancreas[10,11,13,16]. NPY-immunoreactive material is frequently found in noradrenaline containing nerve fibers[10,17,18].
Recent studies have demonstrated the existence of a rich nervous network supplying the endocrine as well as the exocrine portion of the pancreas. In addition to the cholinergic and adrenergic nerves, peptide-containing nerves have also been described[19-22]. In view of this, we have carried out a combined (immunocytochemistry-radioimmunoassay) study, to establish the occurrence, distribution, origin and nature of the reported NPY-immunoreactivity of the rat pancreas.
Materials and methods. 50 male Wistar rats weighing 200–250 g each, were divided into five groups (A, B, C, D, E) of 10 animals each. In group A, surgical parasympathectomy of the pancreas was performed by subdiaphragmatic truncal vagotomy. Mickulitz pyloroplasty was also done in this group in order to avoid gastric distension. Group B underwent Mickulitz pyloroplasty alone. Group C had sham-operation (opening and closure of the abdomen under anesthesia). In group D, sympathectomy was performed pharmacologically, by 6-hydroxydopamine (6-OHDA) (200 mg/kg i.p.), a drug that is known to destroy sympathetic nerves[30]. Group E was used as normal controls.
All the animals were kept on a standard diet for 14 days prior to surgery. After 14 days, groups A and B underwent operation (truncal vagotomy and pyloroplasty, or pyloroplasty alone, respectively). After anesthesia (20 mg/kg phenobarbital i.p.) a midline incision was performed and the posterior and anterior branches of the vagus were recognized below the diaphragm, isolated and cut between two ligatures.